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### Innovation in Medical Imaging To Improve Disease Staging, Therapeutic Intervention, and Clinical Outcomes

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## Review article

# Innovation in medical imaging to improve disease staging, therapeutic intervention, and clinical outcomes

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## HIGHLIGHTS

- Calcification plays an important role in the pathogenesis of atherosclerosis and begins early on in the disease.
- Variable calcification patterns are associated with different histopathological and clinical features.
- Modern imaging strategies allow assessment of morphological coronary calcification as well as the underlying early biological changes and activity of calcification.

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## ABSTRACT

Calcification plays an important role in the pathogenesis of atherosclerosis and begins early on in the disease process. The presence of calcium has long been seen as a surrogate marker of atherosclerosis and is a well-established predictor of cardiac risk. Evidence suggests that different calcification patterns are associated with different histopathological and clinical features. At the patient level, the presence of macrocalcification, as assessed by the coronary calcium score, confers worst outcomes. At the plaque level, microcalcification rather than macrocalcification denotes plaque vulnerability. Improved non-invasive imaging modalities may allow for a more comprehensive assessment of atherosclerotic calcification and help identify patients at increased risk of clinical sequelae.

## 1. Introduction

Calcification plays an important role in the pathogenesis of atherosclerosis and begins early on in the disease process. The presence of calcium has long been seen as a surrogate marker of atherosclerosis and is a well-established predictor of cardiac risk [1]. In recent years, coronary calcification has come under renewed attention, with growing evidence suggesting that different calcification patterns are associated with different histopathological and clinical features.

Traditional computed tomography calcium scoring measures visible calcium deposition in the coronary arteries – otherwise known as macrocalcification. The current thinking is that macrocalcification identifies a high-risk vulnerable patient rather than a vulnerable plaque or vulnerable vessel. On the other hand, microcalcification consists of micro-deposits of calcium (smaller than 50  $\mu$ m), which cannot be detected by conventional CT and is thought to represent the early stages of

the process and may in fact indicate plaque vulnerability. In the transition from microcalcification to macrocalcification, small discrete nodules of calcium (up to 3 mm) appear termed “spotty calcification”. Recent data suggest that plaque rupture events are more common in less calcified lesions with higher degree of local inflammation rather than in densely calcified, healed atherosclerotic plaque. The so called “calcium paradox” is still a topic of considerable debate. Improved non-invasive imaging modalities have shed light on the mechanisms regulating the evolution of atherosclerotic calcification and helped identify patients at increased risk of clinical sequelae.

This review will focus on coronary calcification: the underlying pathogenesis and molecular mechanism, implications with regards to plaque progression and the relationship of the extent and patterns of calcification to plaque morphology. We will explore the established and emerging imaging modalities and the potential implications on diagnosis, risk stratification, and patient care.

**Abbreviations:** VSMC, vascular smooth muscle cell; RANK, receptor activator of nuclear factor kappa-B; BMP, bone morphogenetic protein; OPG, osteoprotegerin; OCT, optical coherence tomography; IVUS, intravascular ultrasound; CAC, coronary artery calcium score; CT, computed tomography; PET, positron emission tomography; 18F-NaF, 18F-sodium fluoride

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## 2. Pathogenesis

Atherosclerosis is an inflammatory process defined by intimal or subintimal lipid deposits forming fatty streaks. Progressive lesions develop a necrotic core with abundant macrophages, foam cells, cellular debris and extravasation of erythrocytes from newly formed fragile capillaries [2]. Throughout this process of plaque development, the composition of the fibrous cap (crucial in determining the plaque's structural integrity) is also in a state of flux. A thick cap is associated with relative plaque stability [3]. However, inflammation via the action of matrix metalloproteinases drives decreased synthesis and increased breakdown of collagen, resulting in thinning and weakening of the cap and increasing the risk of plaque rupture and exposure of its thrombogenic constituents to the blood [4].

The body has several healing mechanisms that seek to stabilise atherosclerotic plaques including calcification. Calcification starts early on in the atherosclerotic process and is observed in lesions with pathological intimal thickening [5]. It is believed to occur as a healing response to intense necrotic inflammation and it is useful to consider the calcification in two stages. It is postulated that microcalcification represents the early stages of the process and is triggered by intense inflammation within the lipid core of the atheromatous plaque [6]. Microcalcifications are associated with markers of plaque vulnerability, such as intraplaque haemorrhage [7], and its presence in the fibrous cap might promote cavitation-induced plaque rupture [8]. In contrast, macrocalcification represents the end stages of disease with the formation of homogeneous or sheet-like calcification which effectively walls off the inflamed necrotic core and stabilises the plaque by serving as a barrier to inflammation and rupture [9].

Pathomorphologically, atherosclerotic calcification typically affects the arterial intimal layers in association with macrophages, lipids and vascular smooth muscle cells (VSMC) [10] which should be distinguished from calcification in arterial medial layers causing elastin mineralisation and subsequent loss of elasticity and is often associated with renal failure, diabetes mellitus, hypercalcaemia, and hyperphosphataemia [11]. The same conditions associated with medial calcification – namely diabetes mellitus and chronic kidney disease – are also associated with accelerated atherosclerosis.

Whilst the exact underlying molecular mechanisms of atherosclerotic calcification are largely unknown, histological studies have highlighted the complexity of the cellular interactions involved in vascular calcification; a process that involves positive and negative regulators that orchestrate cellular recruitment, differentiation, and function [12]. A number of resident and circulating cells are subjected to such processes including mesenchymal stem cells, macrophages and vascular smooth muscle progenitor cells. They have all been shown to undergo osteoclastic differentiation [12]. This process is triggered by two main cytokines: monocyte colony-stimulating factor and the ligand for receptor activator of nuclear factor (NF)- $\kappa$ B (RANKL).

Initial calcification is thought to result from apoptosis of smooth muscle cells: a process triggered by pro-inflammatory cytokines released from local activated macrophages. Calcifying extracellular vesicles are released with formation of micro-deposits of calcium (smaller than 50  $\mu$ m), of which hydroxyapatite is the main component [8,13]. Macrophage-derived matrix vesicles also play a role in the process of microcalcification [14] resulting in larger punctate appearance. Microcalcifications coalesce into a larger mass and become spotty calcification (< 3 mm). These can then go on to coalesce into larger masses forming macrocalcific deposits. This homogeneous or sheet-like calcification effectively walls off the inflamed necrotic core. However, calcified sheets may fracture leading to the formation of nodular calcification thus compromising the continuity of the endothelial lining and underlying collagen matrix [15]. Sugiyama and colleagues have shown that superficial calcification is a prevalent type of macrocalcification in acute coronary syndrome and is associated with greater post-intervention myocardial damage [16].

The biological processes underpinning the transformation from micro-deposits of calcium to more organised stable deposits of macrocalcification are closely linked and, to a degree, regulated by the underlying inflammatory process within atherosclerotic plaque.

### 2.1. Role of inflammation

Vascular inflammation appears to proceed and to trigger the calcification process [17]. Initial calcium deposition in response to pro-inflammatory stimuli results in the formation of granular calcification ("microcalcification"). A positive feed-back loop further stimulates macrophage activation and mineralisation produces foci of calcification which induce further inflammation [18]. Pre-clinical animal studies suggest in the early stages of the atherosclerotic process, vascular inflammation and osteogenesis progressed in close proximity to, and increased in parallel with, plaque progression [19]. Paradoxically, advanced atherosclerotic lesions demonstrated an inverse relation between inflammation and calcification.

In the early stages of atherosclerosis, the M1 subtype of macrophages predominate, and pro-inflammatory cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6) promote early phases of osteogenic differentiation of VSMC and vesicle-mediated calcification as the result of apoptosis of macrophages and VSMCs themselves [20]. It is postulated that this microcalcification represents the early stages of the process and occurs as part of the healing response to intense inflammation within the necrotic core and may in fact prompt a vicious circle of inflammation and calcium deposition [21]. The presence of microcalcification within plaques is associated with larger lipid burden, thinner fibrous cap and higher frequency of microchannels which predispose to plaque rupture [22].

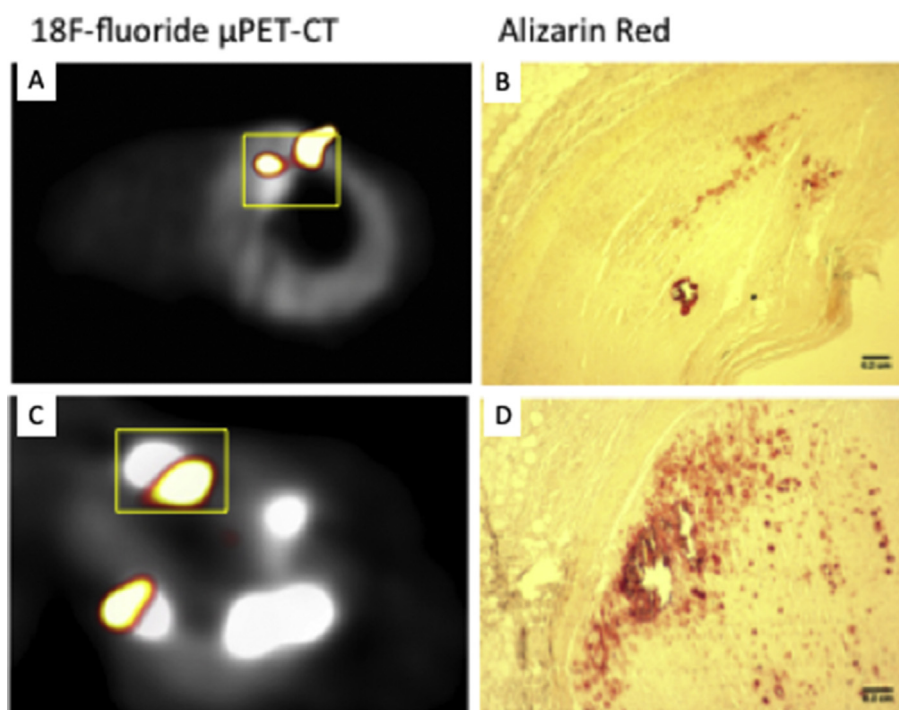
As plaques stabilise, the M2 subtype of macrophages predominate [20]. M2 contribute to inflammation resolution and plaque remodeling. They secrete a number of anti-inflammatory cytokines, such as interleukins (e.g. interleukin-10), which promotes osteoblastic differentiation and maturation of VSMC, which facilitate macrocalcification [23]. In contrast to the aforementioned microcalcification, macrocalcification represents the stable stages of more advanced atherosclerosis with the formation of homogeneous or sheet-like calcification which effectively walls off the inflamed necrotic core, and stabilises the plaque by serving as a barrier to inflammation and rupture.

### 2.2. Molecular proteins, osteoregulation and calcification

Calcification within atherosclerotic lesions has features similar to resorptive and remodelling sites in trabecular bone. The mechanisms by which bone-regulatory proteins influence the pathophysiology of atherosclerotic calcification is the subject of intense interest.

Bone-related proteins - bone morphogenetic protein (BMP)-1 and BMP-4 [24], osteocalcin, matrix Gla protein, osteonectin, osteopontin [25], and osteoprotegerin – have been identified within the vessel wall, regulating the deposition of vascular calcium [26]. Functional matrix Gla protein (MGP), a tissue-derived vitamin K dependent protein, is primarily secreted by vascular smooth muscle cells (VSMCs) in the arterial medial layer and is thought to be a potent inhibitor of bone morphogenetic protein and therefore vascular calcification [27]. Lack of MGP activates BMP signalling throughout the vascular wall and is associated with ectopic osteochondrogenic differentiation, vascular calcification, and endothelial-mesenchymal transitions (EndMTs); a process by which endothelial cells acquire a mesenchymal phenotype and stem-cell like characteristics [28].

Osteopontin, calcium-binding glycoprophosphoprotein, expressed by macrophages as well as smooth muscle and endothelial cells within plaque also appears to inhibit calcification [29]. Plasma osteopontin concentrations are higher in patients with coronary artery disease [30] but were not independently associated with coronary calcification. Histological studies have confirmed the presence of osteopontin, and



**Fig. 1.**  $^{18}\text{F}$ -Sodium fluoride uptake in coronary artery atherosclerotic plaque. Micro-positron emission tomography (PET) and computed tomography (CT) of  $^{18}\text{F}$ -sodium fluoride uptake in a coronary artery plaque (A and C), which colocalizes with active calcification demonstrated by alizarin (red) staining (B and D).

matrix Gla protein at sites of microcalcification early on in the disease process [31]. Meanwhile, fibrocalcific plaques have been found to contain BMP-2, BMP-4, osteopontin, and osteonectin [32].

The osteoprotegerin (OPG), the receptor activator of NF- $\kappa$ B ligand (RANKL) and the receptor activator of NF- $\kappa$ B (RANK) cytokine network regulates balance between bone formation (osteoblasts) and bone resorption (osteoclasts) [33]. Osteoprotegerin (OPG) prevents osteoclast differentiation and bone resorption and exerts its effect through binding and neutralizing RANKL with strong osteoclast-inducing activity [34]. In addition to its osteoregulatory role, OPG is also able to bind and to neutralise the pro-apoptotic actions of tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) expressed by VSMC. It may in fact play an important role in plaque stability [35].

Osteoprotegerin (OPG), which has been shown to be present in atherosclerotic plaques [36], appears to act as an autocrine or paracrine regulator of vascular calcification and may be a useful serum marker of vascular disease [37]. Clinical studies demonstrate increased serum OPG concentrations in association with vascular calcification [36], coronary artery disease [38], cerebrovascular disease and future cardiovascular risk [36]. Some early data suggest that serum OPG concentrations may be an indicator of subclinical atherosclerosis [39]. In patients with established atherosclerotic lesions [40] and angina pectoris [41], serum OPG appears to predict long-term prognosis.

Current studies are somewhat conflicting, and the role of each of these molecular proteins in the diverse morphological manifestations of disease progression is not yet clearly understood. Further prospective trials are needed to confirm this association between osteoregulatory mechanisms and cardiovascular outcome. Drugs that inhibit the RANK/RANKL and bone resorption interaction have yet to demonstrate any cardiovascular benefit.

### 2.3. Calcification and mechanical stress

Mesenchymal cells are maintained in a quiescent state by the surrounding extracellular matrix and regulated by multiple micro-environmental cues. Mechanical stiffness is capable of governing cell

differentiation through activation of intracellular signalling mediators [42], which enhance the osteogenic potential of mesenchymal cells. The resultant calcium deposits can themselves weaken vasomotor responses and alter atherosclerotic plaque stability, depending on the size and distribution of deposits. The shape of a microcalcification is determined by the relationship with collagen and extracellular vesicles. Irregular microcalcification inflicts higher stress than spherical microcalcifications. Large deposits reduce circumferential stress in adjacent plaque [43] and small deposits increase stress at their edges [44]. This is reflected in the different clinical manifestation of coronary calcification: in unstable disease, atherosclerotic lesions demonstrate multiple deposits of “spotty calcification” [45] whereas stable disease is associated with macrocalcification and large calcium deposits [46]. It has been suggested that spotty calcification within the fibrous cap will increase biomechanical plaque stress and increase the risk of plaque rupture [47,48].

### 3. Imaging of coronary calcification

Microcalcification and inflammation play a key role in plaque rupture, therefore representing important potential imaging targets. With modern advances in imaging technology, we now have multiple different techniques to image various aspects of atherosclerotic plaque across different vascular beds. These techniques include invasive imaging using optical coherence tomography (OCT) and intravascular ultrasound (IVUS), and non-invasive imaging with computed tomography, and positron emission tomography, with each modality offering different advantages and disadvantages. Below we discuss how these different imaging approaches can provide a comprehensive non-invasive assessment of atherosclerotic calcification, informing about disease burden, plaque morphology and disease activity.

#### 3.1. Invasive imaging of atherosclerotic calcification

Initial attempts to assess plaque composition and morphology were based around invasive imaging strategies (Fig. 1), predominantly



optical coherence tomography (OCT) and intravascular ultrasound (IVUS), and more recently near-infrared spectroscopy: a novel technique to quantitatively and qualitatively assess lipid cores.

Intravascular ultrasound is an invasive catheter-based technique which uses high-frequency sound waves to generate greyscale cross-sectional images of the arterial wall. It allows the detection and quantification of calcium within a plaque. IVUS appears to provide an accurate quantification of plaque burden, acting as a powerful predictor of disease progression and adverse clinical outcomes [49] and has been used in trials to measure the effect of medical therapies on atherosclerosis [50]. IVUS-identified spotty calcification, defined as calcium deposits within an arc of  $< 90^\circ$ , is most frequently observed in unstable compared to stable plaques (51% vs 30%;  $p < 0.001$ ) [45]. Furthermore, the presence of spotty calcification, is associated with other features of plaque vulnerability namely positive remodelling and fibrofatty plaque [45]. Virtual histology (VH) IVUS uses spectral analysis of ultrasound backscatter to categorise plaque constituents into fibrous, fibrolipidic, calcific, and necrotic tissue in real time [51]. The ability of virtual histology IVUS (VH-IVUS) to detect adverse plaques and then to predict outcomes was investigated in the prospective natural-history study of coronary atherosclerosis (PROSPECT) trial [52].

OCT works on similar principles to IVUS but uses light with a wavelength of about 1-300 nm rather than ultrasound. It has emerged as an insightful intracoronary imaging technology with a higher resolution (10–20  $\mu\text{m}$ ) than IVUS (100–200  $\mu\text{m}$ ). Unlike IVUS, OCT can penetrate calcium and assess its thickness, area, and volume, thus having the potential to provide microstructural detail. It can potentially identify features associated with increased vulnerability such as the presence of macrophages, neovascularization [53], and microcalcifications [54]. The co-localisation of apparent macrophages and microcalcifications in the same plaque is associated with increased plaque vulnerability [55]. In acute coronary syndrome patients, OCT assessment of the culprit vessel highlights a higher prevalence of spotty calcifications at the site of plaque rupture [56], demonstrating the relationship between microcalcification and plaque stability.

Invasive vascular imaging is associated with a risk of complications: 1.6% of the patients in PROSPECT had a complication attributed to IVUS imaging [52]. Furthermore, intra-coronary imaging is unable to image the entirety of the coronary tree, and whilst some basic correlation can be assumed, it cannot assess the overall total plaque burden. Conversely, non-invasive imaging of plaque characteristics across the entire coronary vasculature appears to hold greater clinical potential as a method for identifying patients at increased cardiovascular risk. This is based upon the rationale that patients with a propensity to develop adverse plaque characteristics will do so at multiple sites over time. The vast majority of high-risk thin-capped fibroatheromatous plaques do not result in clinical events [52]. The risk of one such plaque rupturing at an inopportune moment and causing a future clinical event is therefore potentially increased when considered at the level of the patient. Atherosclerosis imaging is accordingly evolving to include not only anatomical but also metabolic imaging, thus providing insight into the underlying vascular biology of patient.

### 3.2. Coronary calcium scoring

Coronary artery calcium score (CAC) measures macroscopic calcification in the coronary arteries and provides an efficient and non-invasive means of assessing and monitoring plaque burden in the totality of the coronary arterial bed. It has repeatedly been shown to correlate with clinical outcomes [57]. In fact, when added to traditional risk score, CAC has the ability to provide incremental risk prediction and appropriately re-classify individuals into higher or lower risk groups [58]. In asymptomatic patients, a calcium score of zero, has a negative predictive value of 95–99% [59]. In these patients, the absence of calcium reliably excludes obstructive coronary artery stenosis.

Coupled with its non-invasive nature, minimal radiation exposure

and no requirement for patient preparation, its powerful predictive ability makes CAC scoring an attractive option for population screening. Current Guidelines recommend CAC scoring in selected patients with a cardiovascular disease risk between 5 and 20% in the context of shared decision-making [60]. Coronary artery calcium scoring should also be considered in patients with cardiovascular disease risk  $< 5\%$  who have a family history of premature coronary heart disease [60]. A coronary artery calcium score  $> 300$  Agatston units (AU) is associated with a four-fold higher risk of cardiovascular events compared to a calcium score of zero [26]. On this basis, the 2013 ACC/AHA Guideline on the Management of High Cholesterol [61] recommended that a CAC score of  $> 300$  AU be used as a modifier to justify statin therapy for primary prevention in adults between 40 and 75 years old without diabetes and with a serum low-density lipoprotein cholesterol concentration 70–189 mg/dL.

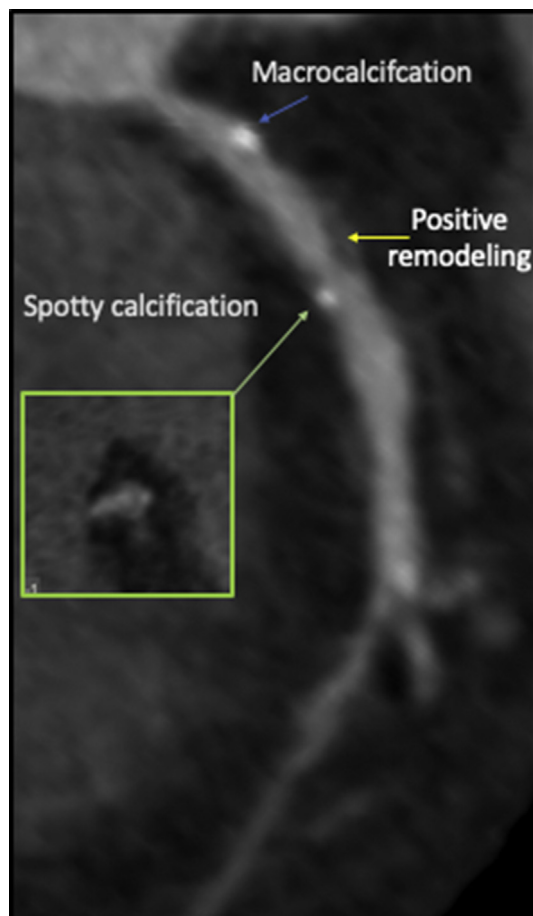
In addition to the traditional Agatston score, routine CAC scoring can also provide information about the density, volume and mass of calcified plaques. There is growing evidence to support the prognostic benefit of coronary density [62], especially in symptomatic patients where it is a stronger predictor of adverse events compared to Agatston score [63]. Whilst traditional calcium scoring remains one of the most powerful prognostic tools, the Agatston score fails to incorporate information about the number and size of calcified lesions and is weighted for increasing calcium with higher calcium density. This is contrary to histological data suggesting that plaques with high calcium density have smaller lipid cores, whilst plaques with low calcium density have large lipid cores and positive remodelling. This highlights an important limitation of CT calcium scoring: namely this approach is actually targeting a more stable form of plaque that itself is less prone to rupture or cause clinical events. This may explain why in symptomatic patients, the presence of coronary calcification correlates poorly with the degree of coronary stenosis [59]. On this basis, the most recent National Institute of Clinical Excellence (NICE) chest pain guidelines recommend coronary CT angiography rather than CAC scoring in symptomatic patients [64]. It is important to consider not only how much plaque a patient has but also what kind of plaque they have and whether the disease process in that area is active or not.

### 3.3. CT assessment of plaque morphology

The last few years have seen rapid growth in the clinical use CT coronary angiography in the diagnosis of coronary artery disease. Our traditional approach to the diagnosis and treatment of coronary disease is centred around the assessment of luminal stenosis. However, there are growing data to support the prognostic power of non-obstructive coronary artery disease [65,66], which is associated with similar event rates to localised obstructive disease [67]. This is consistent with the finding that percutaneous coronary intervention does not reduce the risk of myocardial infarction despite effective relief of obstructive disease and consequent ischaemia [68,69]. Accordingly, there has been growing interest in alternative imaging strategies targeting different aspects of the atherosclerotic disease process.

The sub-millimeter spatial resolution of coronary CT angiography enables imaging of the lumen, as well as the coronary artery wall. At the very least, it is able to differentiate between calcific, partially calcified (mixed) and non-calcified coronary plaque, thereby potentially overcoming an important limitation of CAC scoring [70]. Non-calcified coronary plaques identified by coronary CT angiography confer a poorer prognosis [71,72]. These findings are in line with histopathological studies which have reported that lesions associated with acute coronary events are often not heavily calcified [73,74].

There are several classic CT coronary angiography features of high risk plaque (Fig. 2) which reflect the underlying pathological changes: low-attenuation ( $< 30$  Hounsfield Units), positive remodelling (defined as a remodelling index  $> 1.1$ ), spotty calcification (defined as a calcified plaque component  $< 3$  mm with a  $> 130$  HU density) and the



**Fig. 2.** Mixed types of coronary atherosclerotic plaque on computed tomography coronary angiography. Computed tomography coronary angiogram demonstrating areas of macrocalcification (blue arrow) in the proximal vessel with a further atherosclerotic plaque in the mid vessel with spotty calcification (green arrow) and associated non-calcific positive remodelling (yellow arrow) on the opposing wall.

napkin-ring sign (low-attenuation plaque core with a rim of higher attenuation). There is a large body of non-randomised evidence demonstrating the prognostic value of these findings [75–77]. Recent analyses from the two largest randomised trials of CT coronary angiography in symptomatic patients with suspected stable coronary artery – the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) [78] and Scottish Computed Tomography of the Heart (SCOT-HEART) [79] trials – have added further weight to the

prognostic power of CT coronary angiography assessments of vulnerable plaque. Importantly, approximately half of patients with subsequent adverse events did not have obstructive coronary artery disease [80,81].

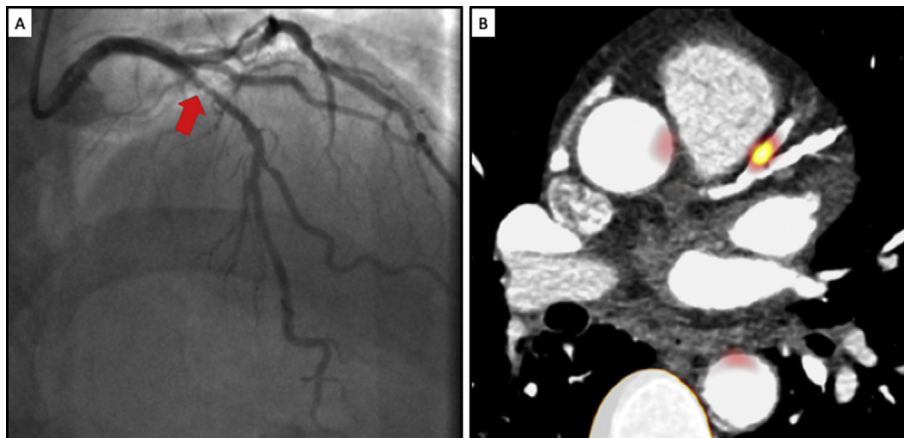
Motoyama and colleagues demonstrated that the presence of spotty calcification was significantly more frequent in the ACS lesions (63% vs. 21%,  $p = 0.0005$ ) [82] and that the presence of high-risk plaque was predictive of future events [76]. In stable patients, CT angiography defined spotty calcification is also associated with adverse outcome (hazard ratio 1.89, 95% confidence interval 1.07–3.33,  $p = 0.0292$ ) [83]. This is in contradiction to a recent prospective study of 245 patients with non-obstructive coronary artery disease on CT angiography which showed that whilst presence of at least two adverse plaque features was associated with a statistically higher rate of cardiac death or acute coronary syndrome (hazard ratio 7.54, 95% confidence interval 2.43–23.34,  $p = 0.0002$ ), spotty calcification alone was not in fact predictive of acute coronary syndrome, all-cause and cardiac death, or very late elective revascularization [84].

The discrepancy in the result of these trials may be caused by differences in patient backgrounds, as well as difficulty in assessment of spotty calcification on CT angiography. Moreover, the limited resolution of coronary CT means it is only able to detect coronary calcifications with minimal diameter of 215  $\mu\text{m}$  [85]. A promising technique for the identification of microcalcifications beyond the resolution limits of CT angiography is  $^{18}\text{F}$ -sodium fluoride positron emission tomography imaging.

### 3.4. Positron emission tomography

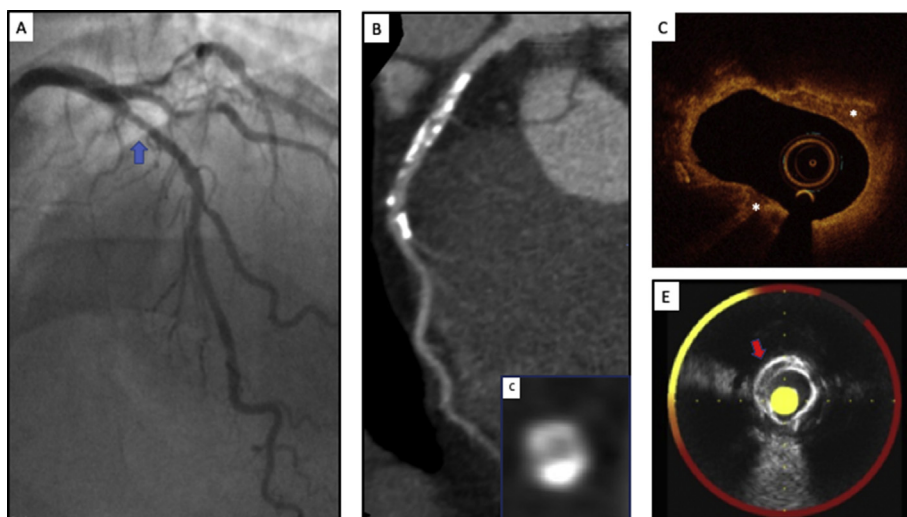
Advances in hybrid scanners now allow combined non-invasive measurement of both disease activity by positron emission tomography (PET) alongside the anatomical detail provided by CT. Targeted PET radiotracers are injected intravenously and accumulate in areas where the disease process of interest is active. The radiation that they emit can then be detected and localised by the PET scanner before being fused with the anatomical data sets.

$^{18}\text{F}$ -Sodium fluoride ( $^{18}\text{F}$ -NaF) is an established radiotracer originally used for the detection of bony metastases and has now found a potential application in hybrid cardiac imaging. It has been used to study vascular calcification activity in a range of conditions including aortic stenosis [86], abdominal aortic aneurysm disease [87] and both carotid and coronary atherosclerosis [88].  $^{18}\text{F}$ -NaF binds to hydroxyapatite through an exchange of fluoride ions with hydroxyl groups where binding is proportional to the surface area of exposed hydroxyapatite [89]. This allows  $^{18}\text{F}$ -NaF to detect active microcalcification area beyond the resolution of CT scan [90]. The increased surface area to volume ratio of micro-calcification relative to macro-calcification results in both increased and concentrated  $^{18}\text{F}$  tracer uptake in the



**Fig. 3.** Culprit plaque imaging in a patient with anterior myocardial infarction.

Invasive coronary angiography showing a culprit stenosis of the left anterior descending coronary artery (red arrow; A) in a patient with an anterior myocardial infarction. Intense focal  $^{18}\text{F}$ -sodium fluoride uptake is observed at the site of the culprit plaque on the combined positron emission and computed tomography coronary angiogram (B).



**Fig. 4.** Multi-modality imaging of atherosclerotic plaque.

Invasive coronary angiography demonstrating a moderate stenosis on the left anterior descending artery (blue arrow; A) with corresponding computed tomography coronary angiography confirming the presence of a heavily calcified plaque in the proximal left anterior descending coronary artery (B and C). Optical coherence tomography (D) demonstrating a sharp luminal border low-signal area (\*) indicating an eccentric calcified plaque and a near-infrared spectroscopy-intravascular ultrasound (E) of the same vessel demonstrating a ring of calcium (red arrow) with signal drop out and corresponding chemogram demonstrating a lipid-rich core burden (yellow arc).

former (Fig. 3). Recent evidence demonstrates an inverse correlation between plaque calcium density and tracer uptake, with lesions at the lower end of the Hounsfield unit coefficient exhibiting greater radioisotope accumulation whilst denser plaque with high calcium score had relatively lower  $^{18}\text{F}$ -NaF uptake [91]. This may explain the lack of correlation between  $^{18}\text{F}$ -NaF atherosclerotic plaque uptake and CAC score observed in high-risk individuals [92]. This suggests that computed tomography evidence of calcification and positron emission tomography evidence of  $^{18}\text{F}$ -sodium fluoride uptake represent two different markers of atherosclerosis. The former appears to be a surrogate marker of total plaque burden whilst the later may represent an active disease process and denote increased vulnerability.

$^{18}\text{F}$ -Sodium fluoride has the potential to act as a marker of disease activity in the coronary vasculature. Several clinical studies have demonstrated uptake to be associated with culprit and high-risk coronary plaque as defined by invasive angiography (Fig. 4), intravascular ultrasound and CT coronary angiography [88,89,93]. Autoradiography of carotid endarterectomy specimens confirms localisation of  $^{18}\text{F}$ -NaF to the site of macroscopic plaque rupture [88]. Following acute myocardial infarction, increased  $^{18}\text{F}$ -NaF uptake was observed within the culprit plaque [88], a finding supported by subsequent smaller studies [94]. In patients with stable disease, increased  $^{18}\text{F}$ -NaF activity localises to plaque with multiple adverse characteristics – including spotty calcification – on intra-vascular ultrasound [88,95]. In peripheral arterial disease, both inflammation, as measured by  $^{18}\text{F}$ -fluorodeoxyglucose uptake, and  $^{18}\text{F}$ -NaF uptake appear to predict subsequent restenosis following angioplasty [96]. Recent data suggest that coronary  $^{18}\text{F}$ -NaF uptake may also predict progression of coronary calcification in patient with established stable multivessel coronary artery disease.

These data suggest that  $^{18}\text{F}$ -NaF positron emission tomography-computed tomography is a potentially valuable tool in cardiovascular risk stratification. The question that remains to be answered is: can  $^{18}\text{F}$ -NaF signals provide additional risk prediction beyond clinical risk factor scores, blood biomarkers, and anatomic imaging? This is currently being addressed by the ongoing perspective PRE18FFIR trial (NCT02278211).

#### 4. Calcification as a therapeutic target

Statins, an established preventative treatment strategy for coronary artery disease [97], appear to increase not decrease the CT calcium score [98,99]. The beneficial effects of statin are attributed to their effect on plaque stabilisation and slowing of plaque progression [49,100]. This is thought to be partly driven by the pro-calcific effects

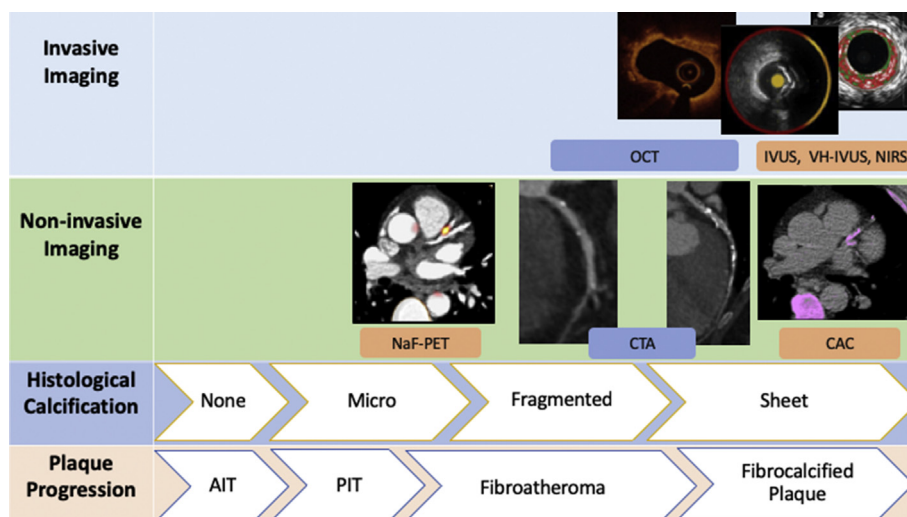
of statin therapy on coronary atheroma that is independent of their plaque-regressive effect [101]. Coronary CT angiography studies have shown that initiation of statin therapy reduces progression of non-calcified plaque volume [102,103]. This may reflect a healing response to statins and highlights a key limitation of CAC scoring, which appears to target a stable form of plaque that itself is not prone to rupture and is unlikely to trigger clinical events. Statins appear to reduce cardiovascular events in conjunction with a reduction in inflammatory markers such as circulating C-reactive protein and pro-inflammatory cytokines [104].

In chronic inflammatory conditions, such as psoriasis and rheumatoid arthritis, higher CAC scores are associated with increased clinical and biochemical markers of active inflammation [105]. It may be reasonable therefore to assume that drugs targeting inflammation, such as tumour necrosis factor alpha antagonists, would result in decreased cardiovascular events. The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) has recently shown that interleukin-1 $\beta$  inhibition confers a reduced risk of athero-thrombotic events [106]. It is unclear whether these cardiovascular benefits are due to an increase in stable coronary plaque calcifications over time.

Warfarin may accelerate coronary plaque calcification, but unlike statin, it may shift atherosclerotic plaques toward a vulnerable phenotype as demonstrated by intimal microcalcifications [107]. In addition to its effects on vitamin K-dependent coagulation factors, animal studies show that warfarin also inhibits vitamin K-dependent extracellular proteins, such as vascular smooth muscle cell-derived matrix Gla-protein (MGP) which normally suppresses calcification of arteries [108,109]. Large clinical trials are needed to assess the effects of long-term warfarin use on clinical events in patients with coronary heart disease and assess its impact on vascular calcification. Similarly, preliminary data suggest that vitamin K supplementation may slow the progression of CAC [110]. However, further research is needed to explore the potential preventative role of vitamin K supplementation in atherosclerotic disease and specifically atherosclerotic calcification.

No study of high-risk plaque identification has yet demonstrated incremental prognostic benefit over and above the total calcium score [79] which remains the most powerful predictor of risk to date. However, evidence relating to the effect of routine medical therapy highlight the calcium paradox and demonstrate that coronary calcium measurements may not accurately reflect the progression of atherosclerotic disease. As previously discussed, the clinical benefit of preventative therapy with statins surpasses their lipid lowering effects and is in contradiction to their impact on the overall calcium score. This raises question regarding whether attenuation of coronary artery calcification progression is a useful therapeutic goal. Can coronary





**Fig. 5.** Atherosclerotic vascular calcification. The relationship between the morphological transformation of coronary calcification and plaque progression highlighting the role of current and emerging imaging modalities at each stage in assess different aspects of coronary calcification. OCT – optical coherence tomography, IVUS – intravascular ultrasound, VH-IVUS – virtual histology intravascular imaging, 18F-NaF-PET – 18F-sodium fluoride positron emission tomography, CTA – CT angiography, CAC – coronary artery calcium score, AIT-adaptive intimal thickening, PIT-pathological intimal thickening.

calcification predicts plaque instability or is merely a marker of plaque burden?

Coronary artery calcification may in fact be protective and may impede further progression of high-risk, low-density plaque. Are current treatment strategies targeting the correct type of calcium? Halting progression of coronary calcification with more intensive modification therapy is perhaps not the most cost-effective way to improve outcomes. Imaging biomarkers targeting the early biopathological steps in atherosclerotic calcification and quantifying active microcalcifications may more accurately reflect the “vulnerable” stage of plaque progression. Large prospective clinical trials comparing their prognostic benefit with established measures of coronary calcification, namely the coronary calcium score, may provide some answers.

## 5. Conclusion

The presence of calcium has long been seen as pathognomonic of atherosclerosis and is a well-established predictor of cardiac risk. Early detection of coronary calcification in younger subjects has important prognostic impact on cardiovascular risk prediction [111]. Rapid advances in non-invasive cardiovascular imaging now allow assessment of the morphological coronary calcification as well as the underlying early biological changes and activity of calcification (Fig. 5). While this has provided important pathophysiological insights, further research is now required to investigate whether these novel approaches provide any incremental clinical information beyond standard patient assessments, and what the impact would be on interfering with the calcific process.

## Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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